Estrogen Receptor-α But Not -β or GPER Inhibits High Glucose-Induced Human VSMC Proliferation: Potential Role of ROS and ERK

Jana Ortmann, Martha Veit,* Sandra Zingg,* Stefano Di Santo, Tobias Traupe, Zijiang Yang, Jan Völzmann, Raghvendra K. Dubey, Stephan Christen, and Iris Baumgartner

Division of Clinical and Interventional Angiology (J.O., M.V., S.Z., S.D.S., Z.Y., J.V., I.B.) and Department of Cardiology (T.T.), Bern University Hospital and University of Bern, and Institute of Infectious Diseases (S.C.), University of Bern, CH-3010 Bern, Switzerland; and Department of Gynecology and Obstetrics (R.K.D.), Clinic for Reproductive Endocrinology, University Hospital Zurich, CH-8032 Zurich, Switzerland

Context: The decreased incidence of cardiovascular disease in premenopausal women has been attributed, at least partially, to protective effects of estrogens. However, premenopausal women with diabetes mellitus are no longer selectively protected. High-glucose (HG) conditions have previously been shown to abolish the antimitogenic effects of 17β-estradiol (E2) in vascular smooth muscle cells (VSMCs).

Objective: Because E2 mediates its action via different estrogen receptor (ER) subtypes, we hypothesized that different subtypes may have different, if not opposing, effects on HG-induced VSMC proliferation.

Methods and Results: Treatment of human aortic VSMCs isolated from premenopausal women with the selective ERα agonist, 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol, but not with E2, the selective ERβ agonist 2,3-bis(4-hydroxyphenyl)-propionitrile, or the selective G protein-coupled ER agonist G-1 completely prevented increased HG-induced VSMC proliferation. Under these conditions, ERα activation selectively prevented increased hydrogen peroxide (H2O2) and total intracellular reactive oxygen species (ROS) production, caused up-regulation of manganese superoxide dismutase protein and activity, and inhibited prolonged ERK phosphorylation. The latter was mediated by ROS, and ROS inhibition reversed HG-induced ERK-dependent VSMC proliferation. The selective coactivation of ERβ reversed the antimitogenic and antioxidative effects of ERα as well as the up-regulation of manganese superoxide dismutase protein expression.

Conclusion: Selective activation of ERα is required for reducing oxidative stress and the consequent hyperproliferation of VSMCs under HG. Our results may further suggest that ERα activation inhibits HG-induced proliferation by down-regulating ROS-mediated ERK activation and may explain why antimitogenic effects of E2 are abolished under HG. Pharmacological activation of ERα may thus have therapeutic potential for treating cardiovascular dysregulation associated with diabetes. (J Clin Endocrinol Metab 96: 220–228, 2011)

Compared with men, women develop cardiovascular disease later in life. This effect has been attributed to the presence of estrogens before menopause and their protective actions on the cardiovascular system (1). However, the loss of cardiovascular protection in premenopausal women suffering from diabetes mellitus suggests that hyperglycemia may abrogate these beneficial effects of estrogens (2). 17β-Estradiol (E2) mediates its effects through different estrogen...

Abbreviations: DCF, 2’,7’-Dichlorodihydrofluorescein fluorescence; DPN, 2,3-bis(4-hydroxyphenyl)-propionitrile; E2, 17β-estradiol; ER, estrogen receptor; FCS, fetal calf serum; G-1, (−)-1-[(3aR*,4S*,9bS*)-4-(6-bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]-ethanone; GPER, G protein-coupled ER; HG, high glucose; MnSOD, manganese superoxide dismutase; NG, normal glucose; PPT, 4,4',4''-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol; ROS, reactive oxygen species; SOD, superoxide dismutase; VSMC, vascular smooth muscle cell.
receptor (ER) subtypes, including the two classical ER subtypes ERα and ERβ, which often mediate differential effects of E₂ (3), as well as by the recently discovered G protein-coupled ER (GPER) (4). It is thus possible that differential activation of ER subtypes is required to promote cardiovascular protection under hyperglycemic conditions compared with normoglycemic conditions or may even cause opposing effects.

High glucose (HG) is known to induce vascular smooth muscle cell (VSMC) proliferation in vitro (5), thus mimicking a key process involved in subendothelial arterial wall thickening and subsequent progression to established atherosclerosis in diabetic patients (6). Although E₂ has been shown to inhibit VSMC proliferation under normal glucose (NG) conditions (7), HG conditions have been reported to abolish the antiproliferative effect of E₂ (8), and the reasons why this occurs are unknown.

VSMCs have been recognized as a major source of reactive oxygen species (ROS) in the vasculature and may thus represent the main source of ROS in advanced atherosclerosis, a situation in which the endothelium is destroyed (9). Furthermore, HG has been shown to increase ROS production in VSMCs (10), which in turn are known to induce VSMC proliferation (11). Specific blocking of oxidative stress in VSMCs may thus represent an important therapeutic approach against vascular disease.

The aim of the present study was to compare the effect of selective and nonselective ER activation on HG-induced VSMC proliferation and to study the potential mechanisms involved. Specifically, we studied the effect of HG on production of H₂O₂ and total intracellular ROS; protein expression of the antioxidant enzyme manganese superoxide dismutase (MnSOD); and phosphorylation of the ERK, a kinase often involved in cell proliferation and sensitive to redox control; and how this is affected by selective activation of ERα, ERβ and GPER vs. nonselective activation by E₂.

Materials and Methods

Cell culture experiments

Human VSMCs isolated from aortic tissue obtained from premenopausal women were purchased from Cascade Biologics (In vitrogen, Basel, Switzerland) and maintained and subcultured according to the supplier’s instructions. Cells were used between passage 3 and 6 only. During these early passages, the phenotype of the VSMCs remains stable, and the response to E₂ is not subject to phenotypic modulation (12). Homogeneity of cells was confirmed by positive immunocytochemical staining for β-actin and negative staining for von Willebrand factor. Cells were cultured in phenol red-free DMEM (Life Technologies, Inc., Sion, Switzerland) supplemented with 10% charcoal-treated, steroid-free fetal bovine serum (dextran coated charcoal fetal calf serum). Phenol red was omitted because it is a weak ER agonist (13). For all experiments, cells were grown to 80% confluency and serum starved for 24 h before treatment. Cells were then restimulated with serum under either normal (NG; 5.5 mM) or high-glucose (HG; 25 mM) conditions and treated with vehicle (ethyl alcohol), E₂ (0.1 μM; Sigma-Aldrich, Buchs, Switzerland), increasing concentrations of the ERα-selective agonist 4,4',4'-4-propyl-[1H]-pyrazole-1,3,5-triyltrisphenol (PPT; 1 nM to 0.1 μM) (14,15), the ERβ-selective agonist 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN; 1 nM to 0.1 μM) (14, 15), or the GPER-selective agonist (±)-1-[(3aR*,4S*,9βS*)-4-(6-bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetraydro-2H-cyclopenta[c]quinolin-8-yl]-ethanone (G-1; 10 nM to 1 μM) (16). All agonists were from Tocris Bioscience (Bristol, UK). To exclude nonspecific effects of the ER agonists, cells were co-treated with 1 μM of the nonselective ER antagonist ICI 182,780 (Sigma-Aldrich) in some of the experiments. To probe for the involvement of ROS and ERK, cells were incubated with the superoxide dismutase (SOD) and catalase mimetic EUK134 (100 μM) (17) and the ERK pathway inhibitor U0126 (10 μM) (18), respectively.

Determination of VSMC proliferation

VSMCs plated in 96-well plates were grown and treated as described above. After 48 h of incubation, plates were washed with PBS, and the amount of cellular DNA per well measured using a CyQuant Cell proliferation assay kit (Invitrogen).

Measurement of hydrogen peroxide

Hydrogen peroxide (H₂O₂) generation by VSMCs was quantified by using the Amplex Red assay (Invitrogen). VSMCs were plated in 96-well plates cultured in DMEM containing 0.1% fetal calf serum (FCS) and treated as described above. After 24 h of incubation, Amplex Red reagent (50 μM N-acetyl-3,7-dihydroxyphenoxazine, 0.1 U/ml horseradish peroxidase in Hanks’ balanced salt solution) was added to each microplate well. After 30 min of incubation at room temperature, H₂O₂ levels were determined by measuring the conversion of Amplex Red using a fluorescence microplate reader set at an excitation wavelength of 530 nm and an emission wavelength of 590 nm and compared with a standard curve prepared with authentic H₂O₂.

Measurement of total intracellular ROS

Cells were grown on glass coverslips and subsequently cultured in DMEM containing 0.1% FCS and treated for 24 h as described above. Subsequently cells were washed with PBS and incubated for 30 min with 5 μM 2',7'-dichlorofluorescein diacetate freshly prepared in dimethylsulfoxide. After washing, cells were counterstained with 4',6'-diamino-2-phenylindole (Invitrogen), 2',7'-Dichlorofluorescein fluorescence (DCF) was examined at 60-fold magnification using an Eclipse 800 fluorescent microscope (Nikon, Tokyo, Japan), and the fluorescence intensity of stained cells was determined using Adobe Photoshop CS4 (San Jose, CA). Fluorescence intensity was expressed as the ratio of the green (DCF) to blue (4',6'-diamino-2-phenylindole) fluorescence to normalize the DCF signal to the cell number.

Western blot analysis for phosphorylated ERK and MnSOD

VSMCs were extracted into radioimmunoprecipitation assay lysis buffer and analyzed as described previously (18). Equal amounts of protein were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA). After blotting onto a nitrocellulose membrane (Macherey-Nagel, Düren, Germany), nonspecific binding sites were blocked for 2 h with milk powder. The membrane was sequentially incubated overnight.
at room temperature with antibodies against phospho-ERK, ERK (both from Cell Signaling Technologies, Beverly, MA; 1:1000), and MnSOD (Stressgen, San Diego, CA; 1:4000). After washing, blots were incubated with horseradish peroxidase-conjugated mouse antirabbit antibody (Sigma; 1:10,000) for 1 h at room temperature. After washing, blots were incubated with peroxidase chemiluminescence substrate (Millipore, Billerica, MA, or Pierce, Rockford, IL) and signals detected on a FluorChem SP imaging system (Alpha Innotech, San Leandro, CA). Band intensities were determined by densitometric analysis using the PC version of NIH Image (Scion Corp., Frederick, MD). Reprobing of the membrane with an antibody against β-actin (Sigma; 1:10,000) was used to verify equal protein loading.

Measurement of MnSOD activity

For the determination of MnSOD activity, we used an SOD activity kit from Assay Designs (Ann Arbor, MI), which is based on a microtiter plate assay using water-soluble tetrazolium salt-1 as indicator for superoxide anion developed by Peskin and Wintemberg (19). VSMCs were plated in six-well plates cultured in DMEM containing 0.1% FCS and treated as described above. After 24 h of incubation, cells were trypsinized and centrifuged at 250 × 1000 g for 10 min at 4 C. The pellet was suspended in 1/10 cell extraction buffer, incubated for 30 min on ice, and centrifuged at 10,000 × g for 10 min at 4 C. One hundred fifty microliters of SOD master mix and 25 μl of 1× xanthine solution was added to each sample in a 96-well plate to initiate the reaction. The MnSOD activity was determined in the presence of 2 mM sodium cyanide (which inhibits copper-zinc SOD >90%), as described previously (20). MnSOD activity levels were determined by measuring the absorbance at a wavelength of 450 nm in a microplate reader and compared with a standard curve prepared with authentic SOD.

Statistical analysis

All data are reported as mean ± SEM. Statistical analyses were performed using Student’s t test or Mann Whitney U test and one-way ANOVA with Bonferroni correction when more than two groups were compared. A P value <0.05 was considered to be significant.

Results

E2 inhibits human VSMC proliferation under NG but not HG

VSMC proliferation was significantly increased under HG conditions compared with NG (150 ± 14%, P < 0.05, Fig. 1A). Although E2 significantly reduced VSMC proliferation under NG conditions, it had no effect on increased VSMC proliferation under HG conditions (Fig. 1A).
Activation of ERα but not ERβ or GPER inhibits increased VSMC proliferation induced by HG

In contrast to E2, treatment with the ERα selective agonist PPT inhibited HG-induced proliferation in a concentration-dependent manner, reaching statistical significance at the highest concentration tested (0.1 μM, Fig. 1B). At this concentration, VSMC proliferation was reduced by almost 50% (from 150 ± 14 to 77 ± 17%, P < 0.01), completely preventing HG-induced proliferation (Fig. 1B). Inhibition of VSMC proliferation induced by HG by PPT was reversed by cotreatment with the nonselective ER antagonist ICI 182,780 (P < 0.01), indicating that the effect of PPT was mediated through ERα and not due to nonspecific effects. In contrast, activation of ERβ or GPER had no significant effect on increased proliferation induced by HG (Fig. 1C and D, P = n.s.).

Lack of effect of E2 on increased H2O2 and total intracellular ROS accumulation induced by HG

To gain insight into the mechanism of the discrepant effect of E2 on VSMC proliferation under NG vs. HG conditions, we determined H2O2 and total intracellular ROS production under the different treatment conditions. Although E2 inhibited proliferation under NG conditions, it had no effect on basal H2O2 production (Fig. 2A), indicating that the antiproliferative effect of E2 under NG is independent of a modulatory effect on H2O2 generation. In line with previous observations (21), incubation of VSMCs under HG conditions caused a significant increase in H2O2 (Fig. 2A) and total intracellular ROS production (Fig. 3). Also, here E2 did not exhibit an inhibitory effect on H2O2 (Fig. 2A) or total intracellular ROS production (Fig. 3).

Selective ERα activation prevents HG-induced H2O2 and total intracellular ROS production

In contrast to E2, HG-induced H2O2 production was significantly inhibited by selective activation of ERα with PPT at a concentration of 0.1 μM (from 188 ± 23 to 124 ± 13%, P < 0.05, Fig. 2B), an effect reversed by cotreatment with ICI 182,780 (Fig. 2B). Whereas GPER activation had absolutely no effect on increased H2O2 production induced by HG (Fig. 2D, P = n.s.), ERβ activation tended to increase H2O2 production, although this effect was not significant (Fig. 2C, P = 0.18).

At a concentration of 0.1 μM, PTT also significantly inhibited total intracellular ROS accumulation (from 224 ± 11 to 120 ± 14%, P < 0.01, Fig. 3) an effect again completely reversed by cotreatment with ICI 182,780. In line with the lack of an inhibitory effect on H2O2 production, although this effect was not significant (Fig. 2C, P = 0.18).

At a concentration of 0.1 μM, PTT also significantly inhibited total intracellular ROS accumulation (from 224 ± 11 to 120 ± 14%, P < 0.01, Fig. 3) an effect again completely reversed by cotreatment with ICI 182,780. In line with the lack of an inhibitory effect on H2O2 production, although this effect was not significant (Fig. 2C, P = 0.18).
tion, neither ERβ nor GPER activation showed a significant effect on increased intracellular ROS accumulation induced by HG (Fig. 3, P = n.s.).

ROS are involved in VSMC proliferation under HG but not NG conditions

The SOD and catalase mimic EUK134 completely prevented HG-induced proliferation (Fig. 4, P < 0.01), whereas it had no effect under NG conditions, indicating that HG-induced proliferation is mediated by ROS.

Selective ERα activation increases protein expression and activity of MnSOD under HG

HG conditions significantly decreased MnSOD protein expression (to 58 ± 16%, P < 0.01, Fig. 5A) and enzyme activity (to 51 ± 6%, P < 0.0001, Fig. 5D) compared with NG. Although not reaching statistical significance, non-selective ER stimulation with E2 tended to restore the decreased MnSOD protein expression (P = n.s., Fig. 5A). However, PPT treatment caused a much higher increase of MnSOD protein expression under HG, e.g. from 58 ± 16 to 220 ± 39% at 0.1 μM (P < 0.01, Fig. 5B). PPT also increased MnSOD activity at the highest concentration tested (from 51 ± 6 to 127 ± 37%, P < 0.05, Fig. 5E); these effects were reversed by ICI 182,780. Selective activation of ERβ had no effect on decreased MnSOD protein expression or activity under HG (P = n.s., Fig. 5, C and F). Furthermore, GPER also showed no effect on MnSOD protein expression (G-1 at 1 μM, P = n.s., data not shown).

Selective ERα activation inhibits the enhanced ERK phosphorylation induced by HG, responsible for increased proliferation

Activation of ERK is known to result in VSMC proliferation and to be modulated by ROS (22). To study whether ERK is potentially involved in HG-induced proliferation and perhaps responsible for the inhibitory effect of ERα activation, we analyzed the status of ERK phosphorylation under NG and HG over time. While under NG conditions, ERK was only transiently activated at 8 h after stimulating starved cells with serum and rapidly declined again, ERK was more persistently activated, i.e. from 8 to 24 h after serum addition, under HG conditions (Fig. 6A).

Inhibition of the ERK pathway by U0126 completely prevented HG-induced proliferation (P < 0.01, Fig. 6B), whereas it had no effect on proliferation under NG conditions, indicating that the increased proliferation induced by HG is mediated by ERK activation. ERK phosphorylation was significantly inhibited by EUK134 under both HG and NG conditions (Fig. 6C), indicating that the activation of ERK in VSMCs under these conditions is redox dependent.

Finally, we were interested to see whether selective activation of ERα is able to inhibit the persistent HG-induced ERK activation at 24 h after serum stimulation, whereas selective activation of ERβ or nonselective activation with E2 does not. Indeed, selective ERα activation significantly decreased enhanced ERK phosphorylation induced by HG (from 137 ± 15 to 48 ± 15% at 0.1 μM, P < 0.05, Fig. 6E), an effect reversed by cotreatment with ICI 182,780 (P < 0.01, Fig. 6E), whereas nonselective ER activation by E2 (Fig. 6D) or selective activation
of ERβ (Fig. 6F) had no significant effect on enhanced ERK phosphorylation. Activation of GPER also did not have an effect on enhanced ERK phosphorylation (G-1 at 1 μM, P = n.s., data not shown).

Coactivation of ERβ reverses the beneficial effects of ERα activation

Because the activation of ERβ showed a tendency to increase H2O2 production, we tested the hypothesis that ERβ may counteract the beneficial effects of ERα activation. Indeed, co-treatment with DPN reversed the protective effects of ERα activation on VSMC proliferation (P < 0.05, Fig. 1B), H2O2 production (P < 0.05, Fig. 2B), intracellular ROS accumulation (P < 0.05, Fig. 3B), and MnSOD protein expression (P < 0.05, Fig. 5B). Co-treatment with DPN also tended to reverse the effects of ERα activation on MnSOD activity (P = 0.14, Fig. 5E) and ERK phosphorylation (P = 0.06, Fig. 6E).

Discussion

In the light of the observation that premenopausal women with diabetes mellitus are no longer selectively protected from cardiovascular disease, despite the presence of potentially beneficial levels of estrogen, we studied the effect of selective ER activation vs. activation with E2 on proliferation of VSMCs isolated from premenopausal women cultured under normal vs. HG conditions. In the present study, we found that although E2 inhibits VSMC proliferation under HG conditions, selective activation of ERα is required to reduce VSMC proliferation under HG conditions. The antiproliferative effect mediated by selective ERα activation was associated with decreased formation of ROS and phosphorylation of ERK, which in turn are responsible for the augmented proliferation triggered by HG. This may suggest that activation of ERα inhibits HG-induced VSMC proliferation by inhibiting ROS-mediated activation of ERK. Activation of GPER did not affect HG-induced VSMC proliferation and ROS formation. Coactivation of ERβ reversed almost all of the beneficial effects of ERα, potentially explaining the lack of effect of E2 on VSMC proliferation under HG conditions (proposed mechanism is summarized in Fig. 7).

In line with previous studies (8), we found that the antimitogenic effect of E2 on VSMCs occurs only under NG but not HG conditions. The differential effect of E2 on VSMC proliferation and H2O2 production offers some insight into the mechanism underlying the differential effect of E2 on VSMC proliferation under HG vs. HG conditions. Thus, the inhibitory effect of E2 on proliferation under NG was not associated with a reduction of H2O2 levels, which have been implicated in VSMC proliferation (11), suggesting that proliferation under HG might not be dependent on ROS. On the other hand, E2 did not have an
inhibitory effect on VSMC proliferation under HG conditions, which we demonstrated to be associated with increased ROS production and thus potentially responsible for the increased proliferation induced by HG in our system. Indeed, the results with the SOD and catalase mimetic EUK134 clearly demonstrate that HG-induced proliferation is mediated by the HG-induced increase in ROS production because EUK134 completely inhibited the hyperproliferative response induced by HG but had no effect under NG conditions. The lack of effect of E2 on VSMC proliferation under HG conditions could therefore be related to the fact that, unlike under NG conditions, ROS are responsible for proliferation under HG conditions.

In contrast to E2, enhanced proliferation induced by HG was completely inhibited by specific activation of ERα. Activation of ERβ and the recently discovered GPER had absolutely no effect on increased proliferation, indicating that only ERα is able to reduce proliferation under HG conditions. The lack of effect of ERβ or GPER activation is not due to a lack of responsiveness of our cells to the selective agonists because ERβ and GPER are expressed in human VSMCs (23, 24). Furthermore, unpublished data of our laboratory show that selective activation of these receptors affects matrix metalloproteinase expression (unpublished observations) and the concentrations of G-1 used in the present study were above the IC50.

The ERα-mediated inhibition of HG-induced proliferation was associated with a reduction of increased HG-induced ROS production. This reduction in ROS production by ERα activation was associated with up-regulation of protein expression and enzymatic activity of MnSOD, an enzyme involved in the protection of vascular cells from oxidative stress (25). Although E2 has been shown to induce MnSOD protein up-regulation (26, 27), the up-regulation of MnSOD protein by specific ERα activation was much higher than that seen by nonspecific activation with E2. ERα has also been shown to be involved in the up-regulation of other antioxidant enzymes (28). These results suggest that MnSOD up-regulation is involved in the reduction of HG-induced ROS formation after specific ERα activation.

ERK is known to be involved in VSMC proliferation (29) and to be induced by ROS (30). Our experiments show that ERK activation during serum-induced proliferation is markedly sustained by HG conditions, suggesting that this prolonged activation might be involved in the increased HG-induced proliferation. Indeed, inhibition of the ERK pathway by U0126 completely abolished the HG-induced increase in VSMC proliferation, whereas it had no effect on basal proliferation under NG conditions. On the other hand, treatment with the ROS inhibitor EUK134 markedly inhibited ERK phosphorylation under both HG and NG conditions, indicating that ERK activation is ROS dependent under these conditions. Like EUK134, activation of ERα, which we have shown to cause a reduction in increased HG-induced ROS production, also significantly reduced prolonged ERK activation. Collectively, these results may suggest that activation of ERα inhibits HG-induced VSMC proliferation by preventing the ROS-mediated activation of ERK (see Fig. 7). However, this association may be circumstantial and in-depth studies are required to further investigate this association.

The tendency of ERβ activation to increase H2O2 formation suggested that ERβ may counteract the beneficial effect of ERα activation on ROS formation and thus on proliferation. Together with the finding that a functional knockout of ERβ increases the cellular resistance to oxidative stress (31), prooxidative actions of ERβ might be a potential explanation for the lack of effect of E2 on HG-induced VSMC proliferation. Indeed, cotreatment of VSMCs with DPN reversed the ERα-mediated effects on H2O2, total intracellular ROS production, and MnSOD protein expression as well as the beneficial effect on hyperproliferation. Opposing effects of ERβ vs. ERα activation have been described previously. Thus, whereas ERα has been shown to mediate the E2-induced up-regulation of genes involved in antioxidant defense, ERβ mediates down-regulation of nuclear genes encoding for mitochondrial electron transport components (28). The two receptors have also been shown to have distinct effects on
proliferation of endothelial cells (32) and aortic (33) as well as coronary artery vasomotion (34).

**Potential clinical implications**

Our findings may, in part, explain why premenopausal women suffering from diabetes are not protected from cardiovascular disease. Thus, in contrary to the inhibitory effect of E2 on VSMC proliferation under NG conditions, E2 had no effect on VSMC proliferation under HG conditions. However, HG-induced VSMC proliferation was inhibited by selective activation of ERα. This beneficial effect was counteracted by concomitant activation of ERβ, indicating that coactivation of the two receptors is not beneficial under HG conditions. Selective activation of ERα may therefore represent a viable therapeutic option for inhibiting VSMC proliferation and improving vascular function (34) under conditions of enhanced oxidative stress. The beneficial effects may affect not only vascular injury and the accelerated progression of atherosclerosis prevalent in diabetic patients but also restenosis rates in both peripheral and coronary arteries after angioplasty.

Because endogenous estrogens play a role for vascular homeostasis not only in premenopausal women but also in young healthy men (35), our findings may also hold potential implications for male patients with cardiovascular disease.

**Acknowledgments**

We thank Corinne Siegenthaler for expert technical assistance and Prof. R. Rieben for support.

Address all correspondence and requests for reprints to: Jana Ortmann, M.D., Division of Clinical and Interventional Angiology, Swiss Cardiovascular Center, University Hospital Bern, CH-3010 Bern, Switzerland. E-mail: jana.ortmann@insel.ch.

This work was supported by Swiss National Science Foundation Grants 310030_120725 (to S.C.), 32-64040.00 (to R.K.D.), and 320000-117998 (to R.K.D.); National Institutes of Health Grant 212-120725 (to S.C.), 32-64040.00 (to R.K.D.), and Grant 310030_120725 (to S.C.) and Prof. R. Rieben for support.

We thank Corinne Siegenthaler for expert technical assistance and Prof. R. Rieben for support.

**References**

15. Harris HA, Katzenellenbogen JA, Katzenellenbogen BS 2002 Characterization of the biological roles of the estrogen receptors, ERα and ERβ, in estrogen target tissues in vivo through the use of an ERα-selective ligand. Endocrinology 143:4172–4177